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Practitioner's Docket No. U 014681-4 ***PATENT***

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Prasad K. **Deshpande**, et al.
Application No.: 10/749,932 Group No.: 1614
Filed: December 31, 2003 Examiner: --
For: BENZOQUINOLIZINE-2-CARBOXYLIC ACID ARGININE SALT TETRAHYDRATE

Commissioner for Patents
P. O. Box 1450
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Attached please find the certified copy of the foreign application from which priority is claimed for this case:

Country: INDIA

Application Number: 915/MUM/2003

Filing Date: September 4, 2003

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JANET I. CORD
(type or print name of practitioner)

Tel. No.: (212) 708-1935

P.O. Address

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c/o Ladas & Parry LLP
26 West 61st Street
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Patent Office
Todi Estates, 3rd Floor,
Lower Parel (West)
Mumbai – 400 013

THE PATENTS ACT, 1970

IT IS HEREBY CERTIFIED THAT, the annex is a true copy of Application and Provisional specification filed on 04/09/2003 in respect of Patent Application No. 915/MUM/2003 of Wockhardt Limited , Wockhardt Towers, Bandra Kurla Complex, Bandra (East), Mumbai – 400 051, Maharashtra State, India, an Indian Company registered under the Companies Act, 1956.

This certificate is issued under the powers vested in me under Section 147 (1) of the Patents Act, 1970.

Dated this 19th day of March 2004.

(N.K. GARG)
ASST. CONTROLLER OF PATENTS & DESIGNS.

FORM 1

THE PATENTS ACT, 1970
(39 of 1970)

APPLICATION FOR GRANT OF A PATENT

[See sections 5(2), 7, 54 and 135 and rule 33A]

1. We, Wockhardt Limited, Wockhardt Towers, Bandra Kurla Complex, Bandra (East), Mumbai 400 051, Maharashtra State, India an Indian Company registered under the Companies Act 1956

2. hereby declare:-

a) that we are in possession of an invention titled 'A Process for Benzoquinolizine-2-Carboxylic Acid Arginine Salt Tetrahydrate'.
b) that the Provisioal Specification relating to this invention is filed with this application.
c) that there is no lawful ground of objection to the grant of a patent to us.

3. further declare that the inventor (s) for the said invention are:

   a) Dr. Noel John de Souza, Dr. Prasad Keshav Deshpande

   b) Wockhardt Towers, Bandra Kurla Complex, Bandra (East), Mumbai 400 051, Maharashtra State, India.

   c) All Indian Nationals

4. We, claim the priority from the application(s) filed in convention countries, particulars of which are as follows :

Not applicable

4627
915

5. I/We state that the said invention is an improvement in or modification of the invention, the particulars of which are as follows and of which I/we are the applicant/patentee:

Not applicable.

6. I/We state that the application is divided out of my/our application, the particulars of which are given below and pray that this application deemed to have been filed on ____ under section 16 of the Act.

Not applicable.

915/MUM/2003
4/9/2003

+ 2000/- Extra
3000/-
4/9/03
4627

7. That we are the assignee or legal representative of the true and first inventors.

8. That our address for service in India is as follows:

Wockhardt Limited
Wockhardt Towers
Bandra-Kurla Complex
Bandra (E)
MUMBAI 400 051
Tel. No. 022-6534444
Fax 022-6534242

9. Following declaration was given by the inventor(s):

We the true and first inventors for this invention declare that the applicant Wockhardt Limited, Wockhardt Towers, Bandra Kurla Complex, Bandra (East), Mumbai 400 051 herein is our assignee.

Dr. Noel John de Souza _____

Dr. Prasad Keshav Deshpande _____

Dated this 3rd day of September 2003

10. That to the best of our knowledge, information and belief the fact and matters stated herein are correct and that there is no lawful ground of objection to the grant of patent to us on this application.

11. Following are the attachment with the application:

a) Provisional Specification – 3 copies
b) Form 2
c) Form 3

We request that a patent may be granted to us for the said invention.

Dated this 3rd day of September 2003

Dr N J de Souza
Director-R&D

To

The Controller of Patents,
The Patents Office Branch, Mumbai.

FORM 2

THE PATENTS ACT, 1970
(39 of 1970)

PROVISIONAL SPECIFICATION
(See section 10)

1. **Title: 'A PROCESS FOR BENZOQUINOLIZINE-2-CARBOXYLIC ACID ARGININE SALT TETRAHYDRATE'**

2. Wockhardt Limited, Wockhardt Towers, Bandra Kurla Complex, Bandra (East) Mumbai – 400 051, Maharashtra State, India, an Indian Company registered under the Companies Act 1956

The following specification describes the nature of the invention and the manner in which it is to be performed.

4 SEP 2003

A PROCESS FOR

BENZOQUINOLIZINE-2-CARBOXYLIC ACID ARGININE SALT TETRAHYDRATE

Field of the Invention

The present invention relates to crystalline S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate and to processes for producing it. Compositions incorporating the tetrahydrate to provide formulations for use in the prophylaxis and treatment of different diseases are also described.

Background of the Invention

S-(-)-9-Fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt is a broad-spectrum antibiotic, medically grouped together with the fluoroquinolone class of antibiotics, which is disclosed and claimed in our U.S. patent 6,514,986 B2 as being isolated in a less crystalline anhydrate form and a more crystalline hydrate form. Our pending U.S. patent application 10/156,685 describes a crystalline monohydrate form of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt that is disclosed as having advantages over the anhydrate and hydrate forms described in US 6,514,986 B2. Such advantageous properties for the crystalline monohydrate form, in comparison to the less crystalline anhydrate and hydrate forms, include enhanced stability at specified conditions of humidity and temperature.

In accordance with the present invention, it has been found that S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate in highly homogeneous form is advantageous over previously known forms and may be usable to prepare stable pharmaceutical dosage forms, including an aqueous solution, because it is the most

physically stable form and does not have a tendency over time to convert to other crystalline forms.

Brief Description of the Drawings

FIG. 1 shows the single crystal X-ray ORTEP diagram of the of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate

FIG. 2 shows the hydrogen bonding network of the water molecules in S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate

FIG. 3 is a X-ray Powder Diffraction (XRPD) pattern of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate.

FIG. 4 is XRPD spectra illustrating conversion of monohydrate to tetrahydrate.

FIG. 5 is a Differential Scanning Calorimeter (DSC) analysis of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate.

FIG. 6 is a thermogravimetric analysis of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate.

FIG. 7 is theoretical XRPD spectrum calculated by a standard software from the coordinates of a single crystal X-ray analysis.

Summary of the Invention

In accordance with the present invention, there is provided a crystalline S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate which is highly homogeneous in regard to other solvatomorphic forms thereof and has superior properties in comparison to such other anhydrate or hydrate solvatomorphic noncrystalline or crystalline forms.

The present invention further pertains to processes for the preparation of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate, pharmaceutical compositions containing it, and the use thereof in the treatment of a wide variety of infections, immune system-related diseases and cancer.

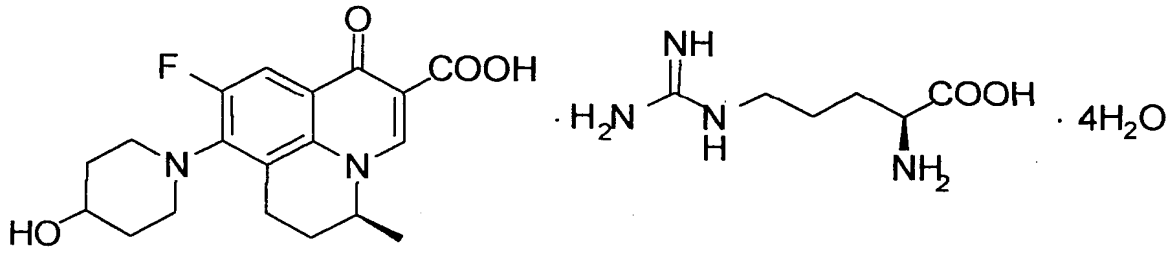
The potent antibacterial activity of the compound of the invention is described in detail below. Additionally, infections due to bacteria begin a chain of immune system events in the host, often invoking inflammation as one of the *in vivo* defense mechanisms. Inflammatory conditions are provoked by the release of inflammatory cytokines acting at various sites. For instance, lipopolysaccharide (LPS) infected mice or mice carrying interabdominal abscesses with Gram-negative *Bacteroidis fragilis* are known to generate macrophage-produced increased levels of the pro-inflammatory cytokine, tumor necrosis factor alpha (TNF-α), which leads to stimulation of endotoxin from the bacterial cell resulting in induced shock and possibly death. Other mediators of the inflammatory immune mechanism may be interleukin-1 (IL-1), interlukin-2 (IL-2), interferron-gamma (IFN-γ) and the like. In the treatment of infectious diseases, often a combination of antibacterial agents and antiinflammatory agents may be used. This use occurs more commonly when the infected host is immunocompromised. In immunocompromised hosts which have lower resistance capabilities, normal bacterial flora and less toxic microbes from the environment may also cause infectious diseases. Such events are usually nosocomial events, often serious and difficult to treat. The combination of antibacterial and antiinflammatory drugs chosen for use in these events should not

have any adverse effect on the host system. Antiinflammatory steroid drugs, however, have a tendency to suppress the immune system and tend to aggravate infectious diseases, while having their own side effects. On the other hand, non-steroidal inflammatory drugs when used with fluoroquinolones may contribute for instance to convulsive effects. Specially those non-steroidal antiinflammatory drugs that are mediators of the GABA-receptor are not good combination partners for a new quinolone antibacterial drug. The S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo [i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate of the invention is a compound that shows potent antibacterial activity against gram-positive bacteria, gram-negative bacteria and anaerobic bacteria, as described more in detail in later paragraphs of this specification. The immunomodulating activity of fluoroquinolones has been reviewed by K. Riesbeck, (Journal of Chemotherapy, 2002, 14 n.1, 3 –12), in which is described their role on phagocytosis, interaction with inflammatory cytokine production by monocytic epithelial and endothelial cells and immunomodulatory effects in animal models. The inhibitory effects on monokine synthesis are suggested to be advantageous in for example gram-negative septicemia and septic shock, while stimulatory effects on bone marrow generation could be important in immunocompromised cancer patients. Bailly et. al., (Int. Journal of Immunopharmc, 1990, 12, 31 – 36) describes that conventional quinolone antibacterial drugs influence formation of inflammatory cytokine from macrophage and monocytes. PCT application WO 02/13830 describes a formulation of gatifloxacin which suppresses the production of cytokine. The compound of the invention in having inherent capabilities for selective modulation of a host's cytokine production makes it useful for the treatment of infection-related immune disorders and suppression of cell-proliferation in cancer.

Detailed Description of the Invention

In accordance with the present invention, there is provided a novel highly homogeneous crystalline tetrahydrate hydratomorphic form of the broad spectrum

antibiotic S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H, 5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt, represented by the following structure:



S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt has been shown in mammals to be effective against a broad spectrum of microorganisms including antibiotic-resistant strains of *Staphylococcus aureus,* more particularly glycopeptide-resistant staphylococci, and to possess excellent overall tolerability.

*Initial methods to prepare S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-*methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt as a bulk active ingredient resulted in an anhydrate solvatomorphic form and a hydrated solvatomorphic form thereof. The physicochemical characteristics of these solvatomorphs are described in U. S. Patent No. 6,514,986 B2. Solvatomorphism is said to exist when a molecule displays an ability to crystallize in different structures that in turn differ in their solvation state (Brittain, Spectroscopy, (2000), 15 (7), 34-39). A hydratomorph may be defined as a solvatomorph in which the solvent is water. Further investigation in the preparation of bulk material revealed that a crystalline form could be produced of a monohydrate of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt, of which the physicochemical characteristics are described in our pending U.S. application No 10/156,685. Even further investigation of the method of preparation for this monohydrate in order to prepare a single crystal of it

provided a hydratomorph which on X-ray crystallographic analysis showed it, surprisingly, to be a tetrahydrate, the detailed data for which is provided below and in the illustrations. An in-depth study of the different hydrates of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt in respect of their respective x-ray diffractograms, differential scanning calorimetry graphs, their water content, their stability order as a function of temperature and/or humidity led to an understanding that the tetrahydrate is the most stable polymorph.

S-(-)-9-Fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo [i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate is further characterized by crystal parameters from single crystal x-ray crystallographic analysis as set forth below.

The crystal of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt is found to form a molecular assembly having a composition of $C_{19}H_{21}N_2O_4F.C_6H_{14}N_4O_2.4H_2O$; a 1:1 complex of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid and L-arginine with four water molecules of crystallization. The salt exists in the zwitterionic form in the orthorhombic system, space group $P2_12_12_1$. The details of data collection, structure solution and refinement are given in Table 1.

Table-1

Single Crystal Parameters of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate.

DATA	Compound of the invention
Formula	$C_{19}H_{21}N_2O_4F.C_6H_{14}N_4O_2.4H_2O$
Formula weight	606.64

Temperature/K	293(2)
Wavelength	MoK_α
Crystal system	Orthorhombic
Space Group	$P2_12_12_1$
a/Å	8.475(5)
b/Å	9.378(6)
c/Å	36.753(3)
$\alpha/^\circ$	90.0
$\beta/^\circ$	90.0
$\gamma/^\circ$	90.0
Volume/$Å^3$	2129.2(3)
Z	4
Density/gcm^{-3}	1.38
Abs. Coeff/mm^{-1}	0.111
F(000)	1407.8
$\theta_{min,\ max}$	2.2, 23.3
$h_{min,max}$, $k_{min,max}$, $l_{min,max}$	-9, 9; -10, 10; -36, 40
No. $Refl^n$. measured	18378
No. unique Reflection	4197
No. parameters	551
Refinement method	Full matrix least square on F^2
R_all	0.062
R_obs	0.041
wR_2 (all)	0.072
wR_2 (obs)	0.067
$\rho_{min,max}/eÅ^{-3}$	-0.132, 0.132
GooF	1.064

The -COOH groups of both S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid and L-arginine are found ionized as $-COO^-$ and H^+. These H^+ ions are taken up by the nitrogen lone pairs of L-arginine to form NH_3^+ and NH_2^+ respectively. The four water molecules were found to form strong hydrogen bonds with the $-COO^-$ groups (Fig.2). In fact, H71 of one of the water molecules forms a strong O-H...O hydrogen bond with the $-COO^-$ group (H71...O2 in Fig.2) of the benzoquinolizine-2-carboxylic acid, along with yet another strong O...H-N hydrogen bond with $-NH_3^+$ group of L-arginine (O7...H31 in Fig.2). Thus, this water molecule (O7) bridges the two moieties resulting in a stronger association. However, the other three water molecules, two of which generate a strong O-H...O hydrogen bonds with the $-COO^-$ group of L-arginine (O9H01...O5 and O10H102...06), and the remaining water molecule with $-COO^-$ of the benquinolizine-2-carboxylic acid (O8H81...O2), are less tightly bound, all bindings being shown in Fig.2. A Differential Scanning Calorimetric study (cf. Fig. 5) and Thermogravimetric Analysis (cf. Fig. 6) of the salt of the invention confirms the nature of binding, by three water molecules (O8, O9 and O10) being lost on heating at 70 °C, initially generating a monohydrate by retaining O7.

Crystalline S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H, 5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt tetrahydrate with the water molecules bound as depicted by the single crystal x-ray analysis may be prepared in high homogeneity by the slow evaporation of the clear solution made by dissolving S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo [i,j] quinolizine-2-carboxylic acid L-arginine salt in acetone and water (8:2) mixture.

Fig.3 shows the X-ray diffraction pattern of the salt of the invention.

The present invention also relates to a process for preparing the novel crystalline form of the arginine salt tetrahydrate. A process for the manufacture of the crystalline form of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]

quinolizine-2-carboxylic acid L-arginine salt tetrahydrate comprises the following consecutive steps:

a) heating a suspension of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt in an organic solvent such as acetone, acetonitrile preferably acetone and water at 70-80^{0}C to obtain a clear solution;
b) cooling the solution to provide a crystalline substance;
c) isolating the crystalline form of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate at 30^{0}C - 35^{0}C by filtration or centrifugation;
d) air drying of the crystalline form of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate at a temperature between 30^{0}C - 35^{0}C.

Of the various crystalline forms of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt, the primary one that crystallizes directly from aqueous acetone solutions is the tetrahydrate. This tetrahydrate shows on Karl Fischer (KF) analysis a water content of 11.87 %, (calculated for $4H_20$: 11.88%), equivalent to four molecules of water of hydration. On further drying for 16 hrs at 70 ^{0}C and under high vacuum of 1 mm of Hg the tetrahydrate analyses for a water content of 4.0 %, (calculated for 1 H_2O: 3.98%).

This monohydrate is highly unstable and rapidly absorbs moisture under ambient conditions of temperature (35 ^{0}C) and relative humidity (60 %). Within 20 minutes the XRPD spectra of the monohydrate undergoes changes as illustrated by the diffractograms in Figure 4, wherein the characteristic 2 θ peaks of the monohydrate appearing at 5.28 and 10.66 in Figure 4-A shift to a reduction of the intensity of

these peaks in Figure 4-B with a concomitant appearance of peaks at 2 θ values of 4.84 and 39.2 which are characteristic of the tetrahydrate. In Figure 4-C the total disappearance is seen of the peaks of the monohydrate. Its conversion to the tetrahydrate may be confirmed by the increase in the intensity of the 2 θ peaks at 4.84 and 39.42, peaks which overlap with those in Figure 3 obtained for an authentic sample of the tetrahydrate.

The high homogeneity of the prepared tetrahydrate may be confirmed by comparison of its XRPD spectrum (Fig.3) with that obtained theoretically for single phase tetrahydrate material (Fig.7) by inserting the coordinates derived from a single crystal X-ray structure into a standard software programme. The XRPD spectrum in Fig.3 is seen to be identical with that provided in Fig. 7.

Formulation of the thermodynamically most stable form is a reasonable expectation for a solution mediated process. Using the most stable form rather than a metastable form is advantageous regarding physical stability of the crystalline form. The increased physical stability will afford additional advantages in formulation.

It has been found in accordance with the present invention that the advantageous stability and solubility properties of the tetrahydrate of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt can be applied to the formulation of pharmaceutical dosage forms. Tables providing stability and solubility data are included in the examples. The tetrahydrate of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j] quinolizine-2-carboxylic acid L-arginine salt can be used to prepare aqueous dosage forms. It can also be used to prepare tablets by wet granulation; it can also be formulated by conventional dry granulation.

The dosage forms can be prepared by any conventional techniques recognized in the art, but would preferably be formulated by mixing the tetrahydrate salt of the invention with the other ingredients. The other ingredients utilized to formulate solid oral dosage forms would include conventional inert ingredients such as

microcrystalline cellulose, methyl cellulose and the like, suitable sweetening and/or flavouring agents, and preservatives thereof if required.

Such solid oral dosage forms or dry formulations suitable for the preparation of suspensions would be formulated such that they would contain an effective dose of the compound of the invention. In general, solid dosage forms containing 200 mg - 800 mg of the compound of the invention are contemplated. Preparations suitable for oral suspension would contain a similar dosage.

Pharmaceutical formulations can be formulated together with auxiliaries and additives usually employed in pharmacy, such as tablet binders, fillers, preservatives, tablet disintegrating agents, flow regulating, agents, plasticizers, wetting agents, dispersing agents, emulsifiers, solvents, pH altering additives, flavourings and the like.

The total daily dose range is generally from about 200 mg to about 1500 mg of the arginine salt form. However, the dose may be higher or lower depending on the needs and conditions of the patient.

The following detailed examples serve to more fully illustrate the invention without limiting its scope. It is understood that various other embodiments and modifications in the practice of the invention will be apparent to, and can be readily made by, those or ordinary skill in the art without departing from the scope and spirit of the invention as described above. Accordingly, it is not intended that the scope of the claims appended hereto be limited to the exact description set forth above, but rather than the claims be construed as encompassing all of the features of patentable novelty that reside in the present invention, including all of the features and embodiments that would be treated as equivalents thereof by those skilled in the relevant art. The invention is further described with reference to the following experimental work.

Example 1

Preparation of the single crystal of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt terahydrate.

S-(-)-9-Fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt (1.0 g) was dissolved in a mixture of acetone (40 ml) and water (10 ml) by heating the suspension at 70 ^{0}C for 15 minutes. The clear solution thus obtained was left for slow evaporation at room temperature in a beaker covered with a perforated aluminum foil. The crystal formation started after 2 days. Finally the single crystal was selected for X–ray crystal analysis from a cluster left after complete evaporation of the solvent. The ORTEP diagrams are described in Figures 1 and 2.

Example 2

Larger scale preparation of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt terahydrate

To a three-necked round bottom flask fitted on oil bath and equipped with magnetic stirrer and reflux condenser; S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt (20.0 gm, 55.55 mmoles) was charged in a mixture of acetone (100 ml) and water (25 ml). The reaction mixture was slowly heated under stirring at 70 ^{0}C temperature to obtain a clear solution. The solution was allowed to cool to 30^{0}C - 35 ^{0}C, a crystalline solid obtained was filtered and air dried at a temperature between 30^{0}C - 35^{0}C to afford the title compound 23.5 gm, (80 %). The moisture content by Karl Fisher titration was found to be 11.87% (required for tetrahydrate: 11.88%). The XRPD, DSC, TGA data were determined as described in the Test Examples provided below. The results obtained are described in Figures 3, 5, 6.

Test example-1

Single crystal X-ray analysis

The room temperature single crystal X-ray diffraction data on a prism shaped single crystal were collected on a Bruker AXS single crystal X-ray diffractometer using SMART APEX CCD detector at room temperature [293(2)°K]. The X-ray generator was operated at 50 KV and 35 mA using MoK_α radiation. Data were collected with a ω scan width of 0.3°. A total of 606 frames per sets were collected in three different settings of φ (0°, 90° and 180°) keeping the sample to detector distance of 6.03 cm and the 2θ-value fixed at -25°. The data were reduced by SAINTPLUS [Bruker. SMART, SAINT, SADABS, XPREP, SHELXTL. Bruker AXS Inc. Madison. Wisconsin, USA. 1998] and an empirical absorption correction was applied using the package SADABS [Bruker. SMART, SAINT, SADABS, XPREP, SHELXTL. Bruker AXS Inc. Madison. Wisconsin, USA. 1998]. All the structures were solved using SIR92 [Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. SIR92 - A program for crystal structure solution. *J. Appl. Crystallogr.* 1993, *26*, 343.] and refined using SHELXL97 [Sheldrick G. M. SHELXL97, Program for crystal structure refinement, University of Göttingen, Germany. 1997.].

Molecular and packing diagrams were generated by ORTEP32 [Farrugia, L. J. *J. Appl. Cryst.* 1997, *30*, 565.] and CAMERON [Watkin, D. M.; Pearce, L.; Prout, C. K. CAMERON - A Molecular Graphics Package. Chemical Crystallography Laboratory, University of Oxford, England. 1993.] present in the WINGX (Version 1.64.03b) [Farrugia, L. J. WINGX. *J. Appl. Cryst.* 1999, *32*, 837.] program suite. The geometric calculations were done by PARST95 [Nardelli, M. *J. Appl. Cryst.* 1995, *28*, 569.] and PLATON97 [Spek, A. L. *Acta Crystallogr. Sect A* 1990, *46*, C34.].

The ORTEP diagram of the single crystal (Fig. 1) shows the four water molecules.

The product was analysed for moisture content (12.37 %) by KF titration.

Test example-2

Powder X-ray Diffraction Analysis

300 mg of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo [I,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate prepared as in Example 1 was thinly spread on a sample holder. X-ray diffraction analyses (40kv x 40 mA Rigaku D/max 2200) were performed under the conditions listed below:

Scan speed 5°/ min or 20°/ min

Sampling time 7 min or 2 min

Scan mode: continuous

2θ/θ reflection

Cu target (Ni filter)

The X-ray powder diffraction (XRPD) spectra of the title compound is shown in Fig. 3.

The X-ray powder diffraction (XRPD) spectra of the monohydrate, the tetrahydrate and a mixture thereof is shown in Fig. 4.

Test example-3

Differential Scanning Calorimetry

The Differential Scanning Calorimetry (DSC) was recorded on METTLER TOLEDO STAR system. 5 to 6 mg of the sample was weighed into the aluminum pan, which was then press sealed with an aluminium lid. After three tiny needle holes were made on the lid the sample was tested by heating from 30 °C to 300 °C at a rate of 10 °C/min. The differential scanning calorimetry (DSC) analysis of the title compound is shown in Fig. 5.

Test example-4

Thermogravimetric analysis

Thermogravimetric Analysis (TGA) was recorded on a METTLER TOLEDO TGA/SDTA 851 system. 5 to 10 mg of the sample was weighed into the aluminum pan and sample

was tested by heating from 30 °C to 300 °C at a rate of 10 °C/min.
The thermogravimetric analysis (TGA) of the title compound is shown in Fig 6.

Test example-5

pH-Related solublility of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo [I,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate at 30 °C

An accurately weighed amount about 20 mg of the compound was transferred to a conical flask and a buffer solution of appropriate pH was added in portions (0.2 ml at a time) till a clear solution was obtained. At buffer pH values of 8.0 and 8.5 addition of buffer was discontinued at 20 ml.

pH of buffer	Solubility (mg/ml)
8.0	< 1.0
8.5	< 1.0
9.0	2.0
9.5	5.0

Test example-6

Temperature/Relative Humidity-related stability of S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo [I,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate

3 Sample batches of the compound were stored under the conditions of the study as stated in the table below. The assay was done by a validated HPLC method. The results are provided in the table below.

Temp.(°C) /Relative Humidity (%)	Time (months)			
	Initial	1	2	3
40 / 75	99.31 %	99.30 %	99.25%	99.27%
25 / 60	99.31%	-	-	99.22%

The crystallinity of the substance underwent no changes as seen by XRPD spectra.

Test example-7

Effects of the compound of the invention on IL-2 production by lymphocytes.

The compound of the invention (conc. range 0.3125 to 80 □g/ml) was tested in a cell. system consisting of peripheral blood lymphocytes (PBLs). PBLs isolated from buffy coats or from heparinized blood from healthy donors were incubated at a density of 10^6/ml. To quantify DNA synthesis, PBLs were pulsed with [*methyl*-^{3}H]thymidine (1 □Ci) during the final 18 hrs of incubation. Thymidine incorporation wasanalysed at two time points (72 and 96 hrs of culture). From known correlations between interleukin-2 (IL-2) and thymidine incorporation, increased thymidine uptake observed in the present compound of the invention as compared to drug-free controls, the IL-2 synthesis was nalysed using a specific ELISA.

Test Example-8

Screening for activity against several cancer cell lines

The compound of the invention was screened according known protocols.

ABSTRACT

The invention relates to crystalline S-(-)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate, a process for its preparation and pharmaceutical formulations incorporating it as the active ingredient for use in treating microbial infections.

Dated this 3rd day of September 2003

Dr. N J de Souza
Director-R&D

04 SEP 2003

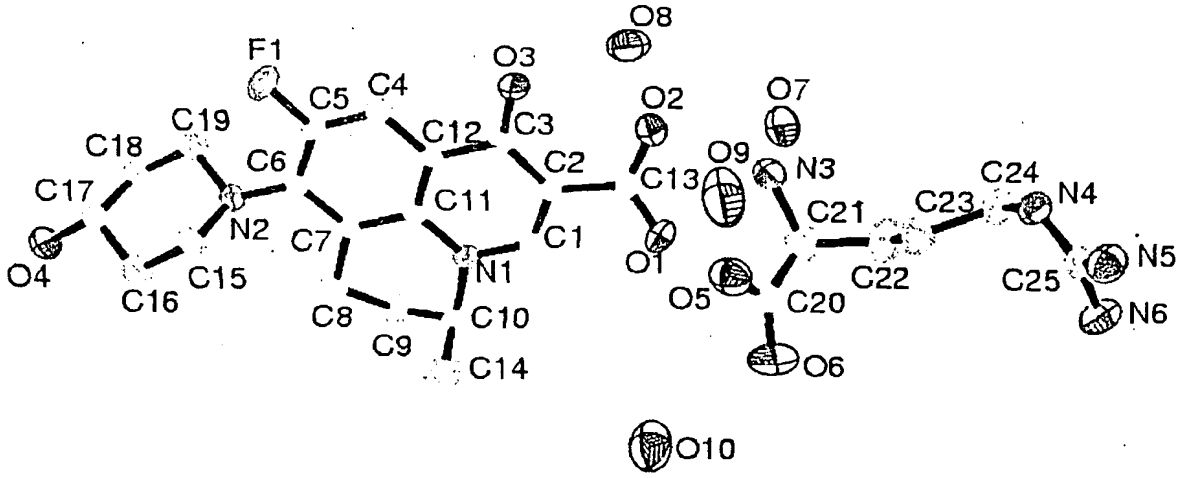


Fig.1

ORTEP View of the Molecular Structure with numbering system

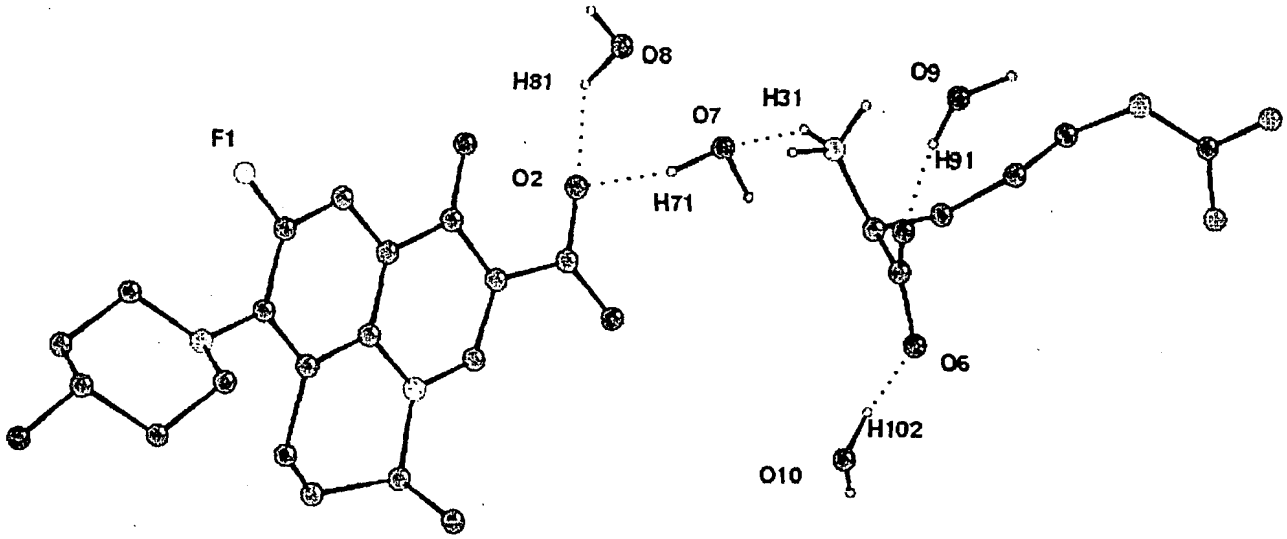


Fig.2: Hydrogen bonding of the water molecules

FIG. 3 : X-ray Powder Diffraction (XRPD) Spectrum of the tetrahydrate of the invention.

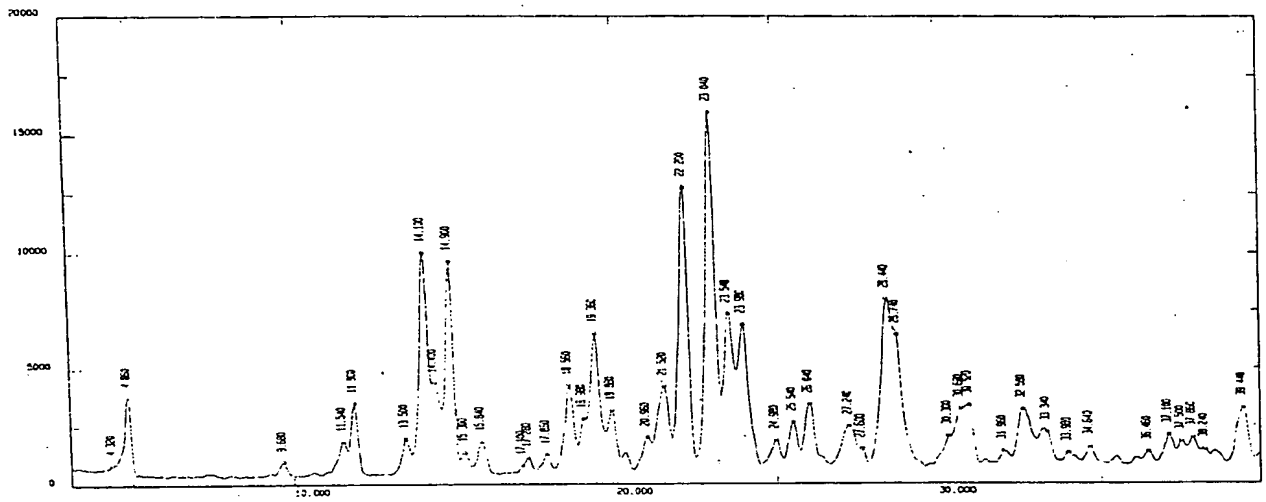


FIG. 4 : XRPD Spectra of conversion of monohydrate to tetrahydrate

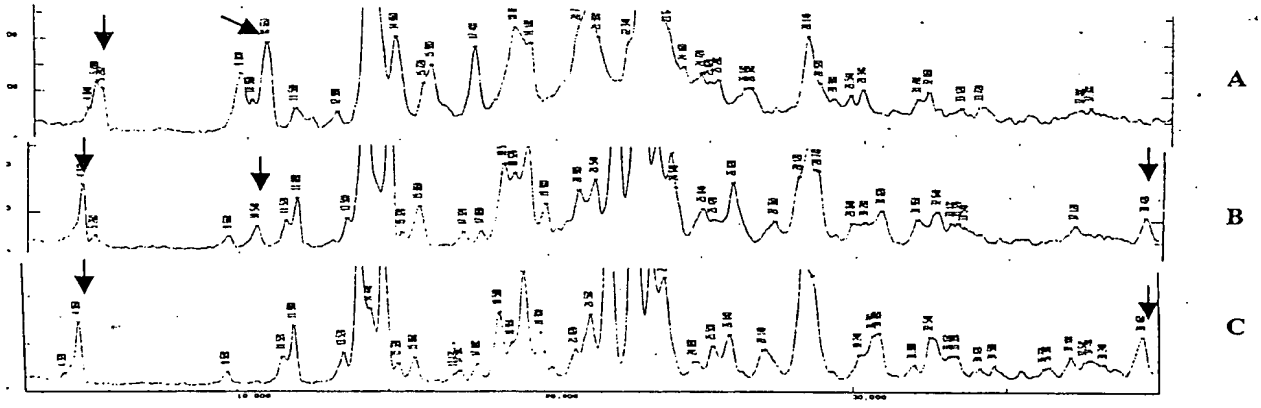


FIG. 5 : Differential Scanning Calorimeter (DSC) Analysis of the tetrahydrate of the invention.

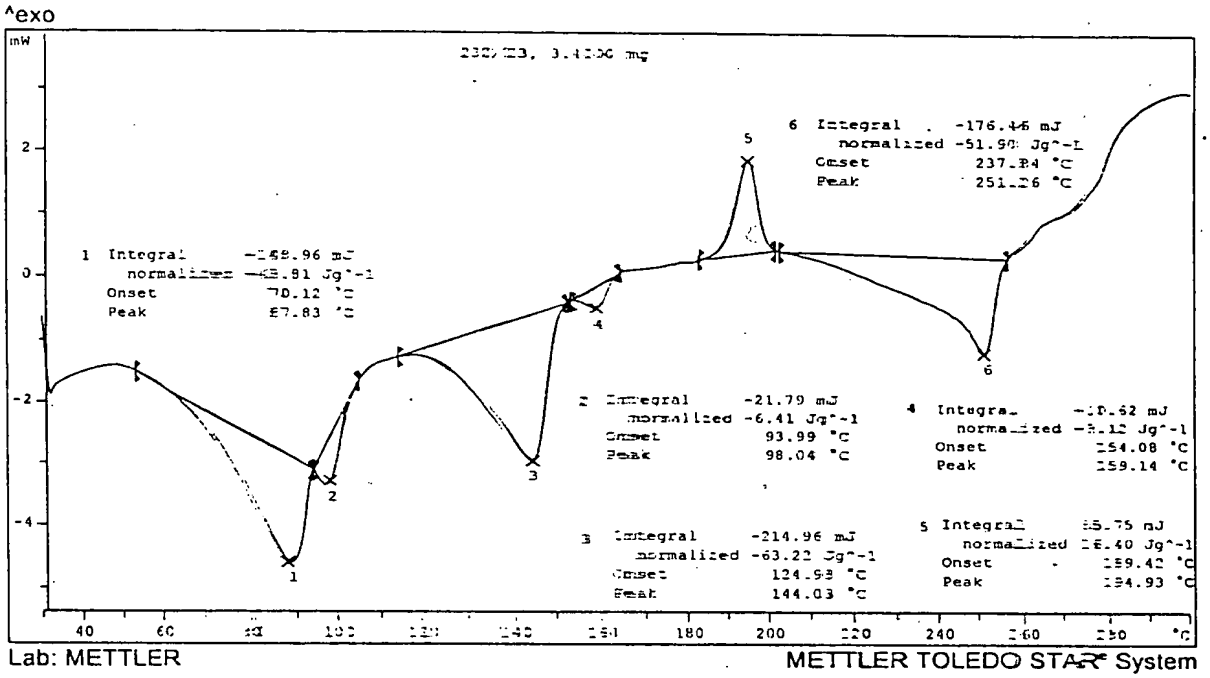


FIG. 6 : Thermogravimetric Analysis (TGA) spectrum of the tetrahydrate of the invention.

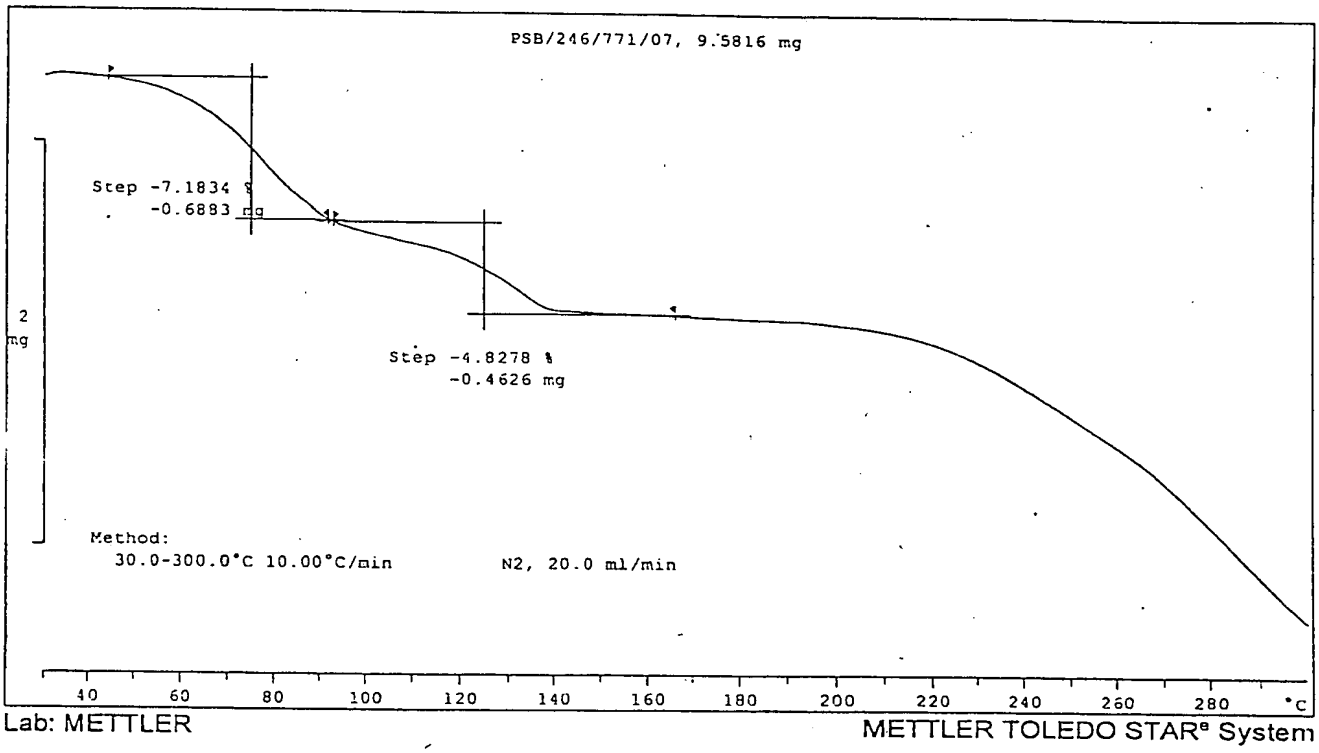


FIG. 7 : Theoretical XRPD spectrum of a tetrahydrate salt of the invention